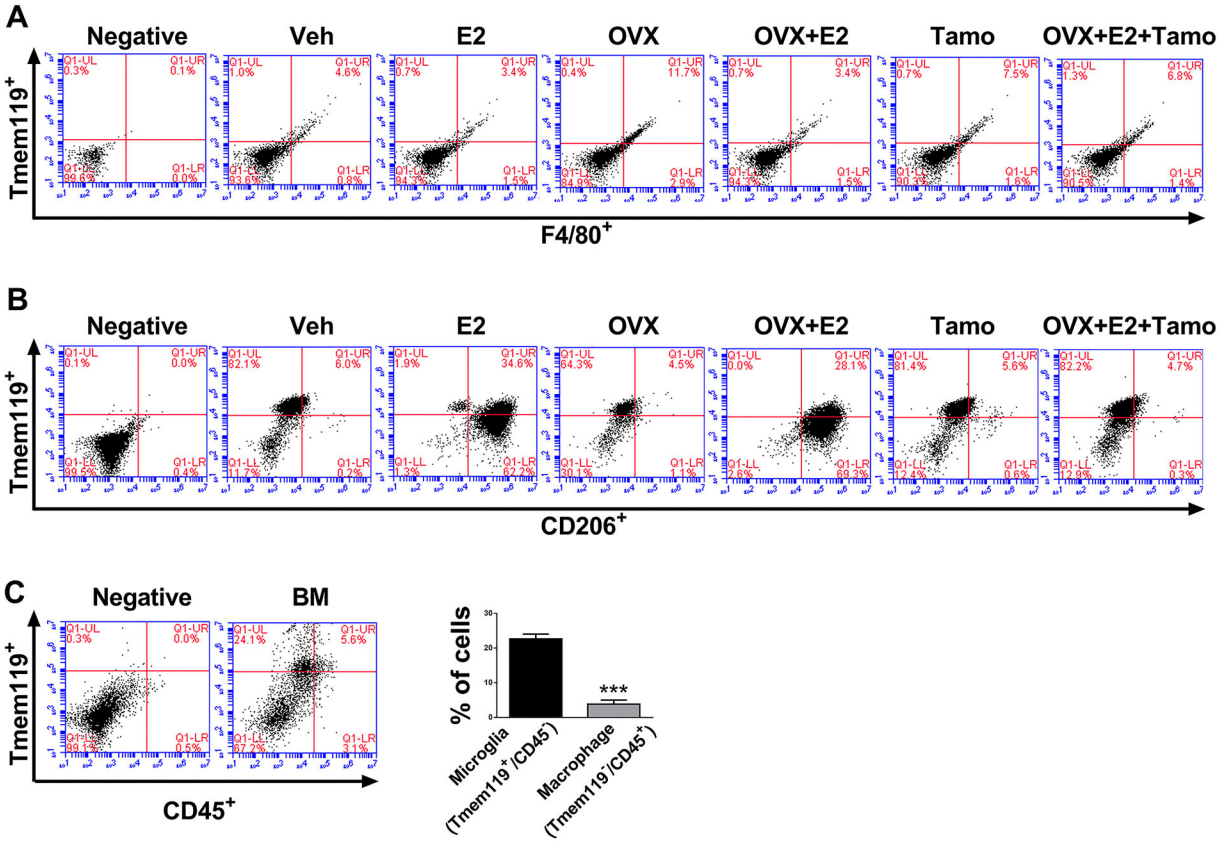
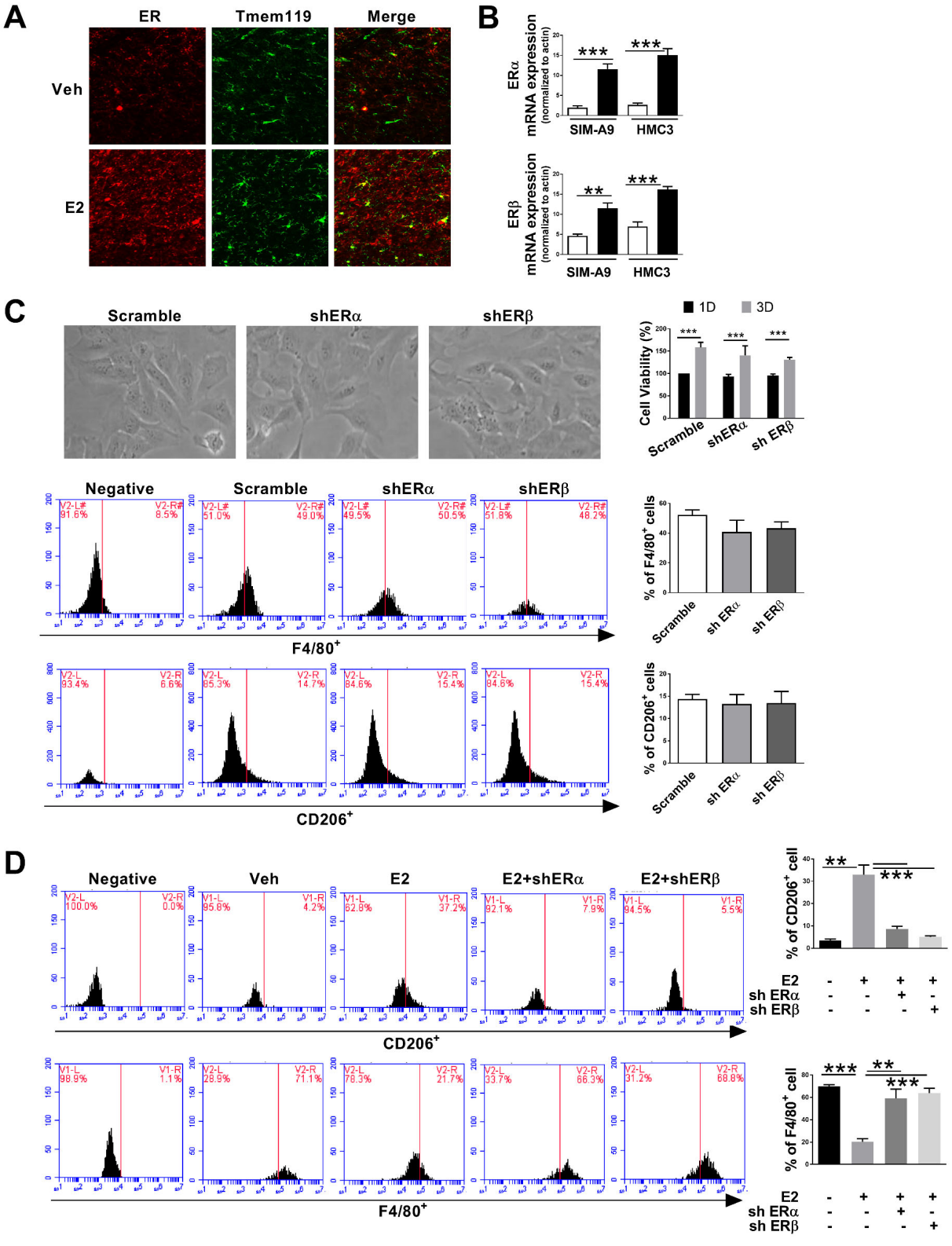


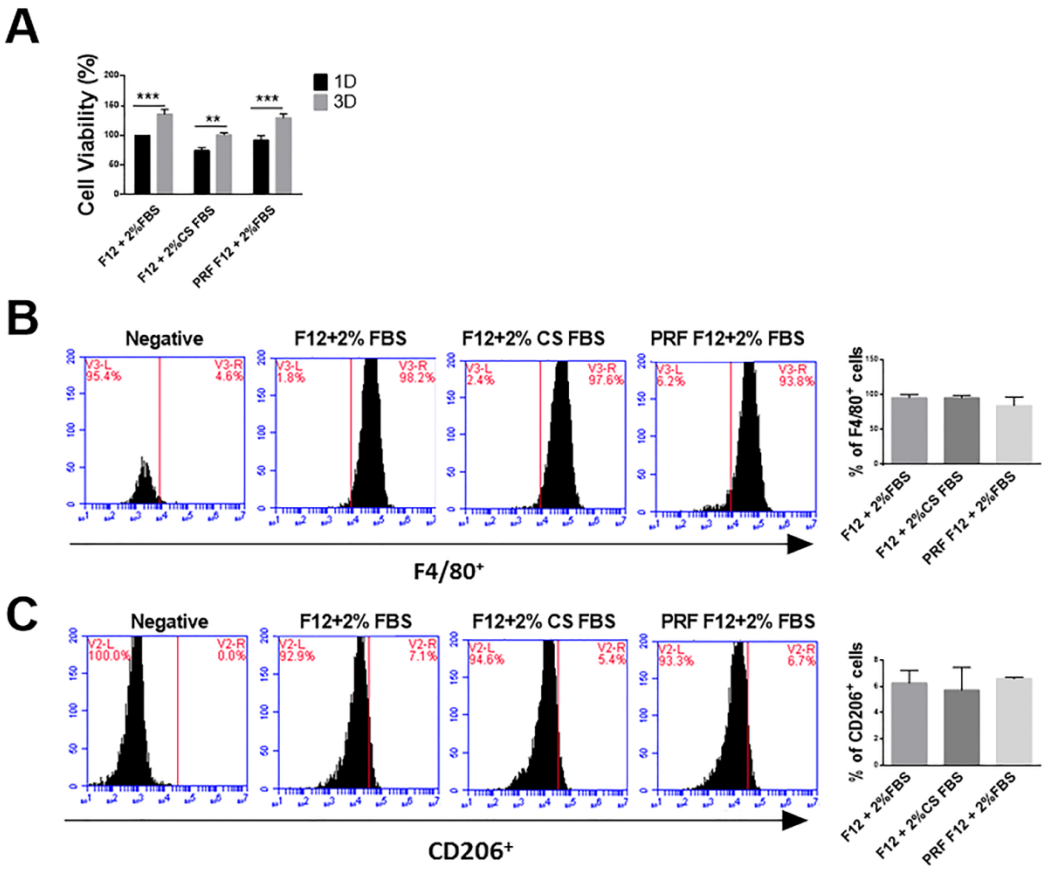
Supplementary Fig 1: Estrogen promoted M2 microglial polarization.

A and **B.** Metastatic brain tumors from Figure 1A were isolated and examined by flow cytometry for M1 ($F4/80^+/Tmem119^+$) and M2 ($CD206^+/Tmem119^+$) microglia polarization. **C**. Infiltration of microglia and macrophage in mouse brain in 231BrM transplanted mouse brain (n=3). *t-test*, ***: $p < 0.001$. The data are presented as the mean ± SD.


A
ER
Tmem119
Merge
Veh
E2
B
ERα mRNA expression (normalized to actin)
SIM-A9
HMC3
ERβ mRNA expression (normalized to actin)
C
Scramble
shERα
shERβ
1D
3D
Cell Viability (%)
Negative
F4/80+
% of F4/80+ cells
CD206+
% of CD206+ cells
sh ERα
sh ERβ
D
Veh
E2
E2+shERα
E2+shERβ
% of CD206+ cell
% of F4/80+ cell

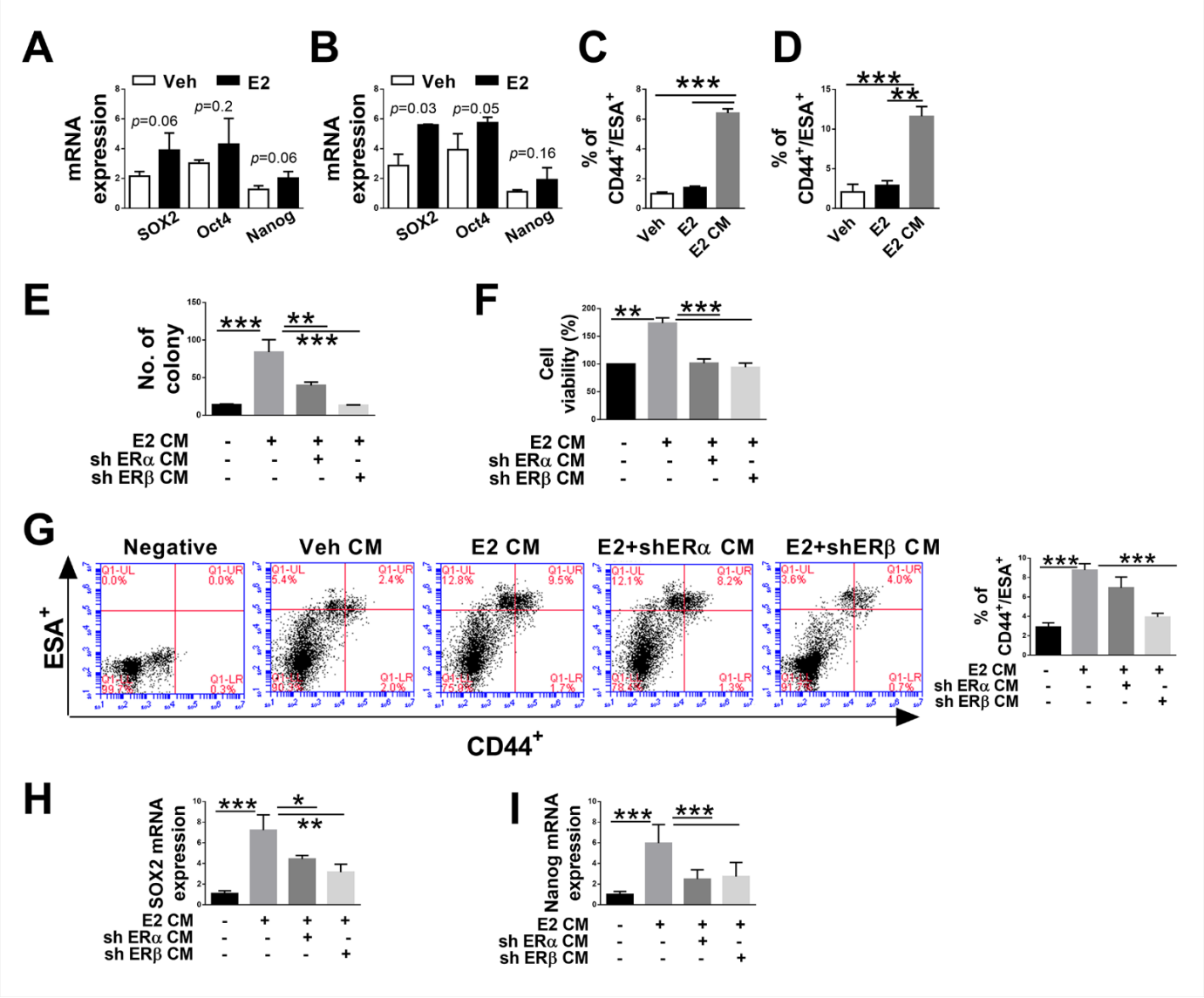
Supplementary Fig 2: Blocking estrogen receptors (ERs) by shRNA suppressed E2-related M2 microglia polarization.

A. Expression of ER on microglia in the mouse brain after 231BrM transplantation with or without E2 treatment. **B.** Mouse (SIM-A9) and Human (HMC3) microglia cells were treated with or without E2 (1 nM) for 24 hours followed by measuring the ERα and β by qRT-PCR. *t* test, **: $p < 0.01$; ***: $p < 0.001$. ($n = 3$). **C.** Morphology, cell viability and M1/M2 polarization of human microglia (HMC3) cells with or without shRNAs treatment. *t* test for cell viability, ***: $p < 0.001$. ($n = 3$). **D.** Population of M1 ($F4/80^+$) and M2 ($CD206^+$) cells were examined by FACS after E2 (1 nM) or E2 plus shERα/β treatment. The data are presented as the mean ± SD. One-way ANOVA, **: $p < 0.01$; ***: $p < 0.001$. ($n = 3$).



Supplementary Fig 3: Effect of phenol red free culture medium and charcoal-stripped fetal bovine serum on cell viability and microglia polarization.

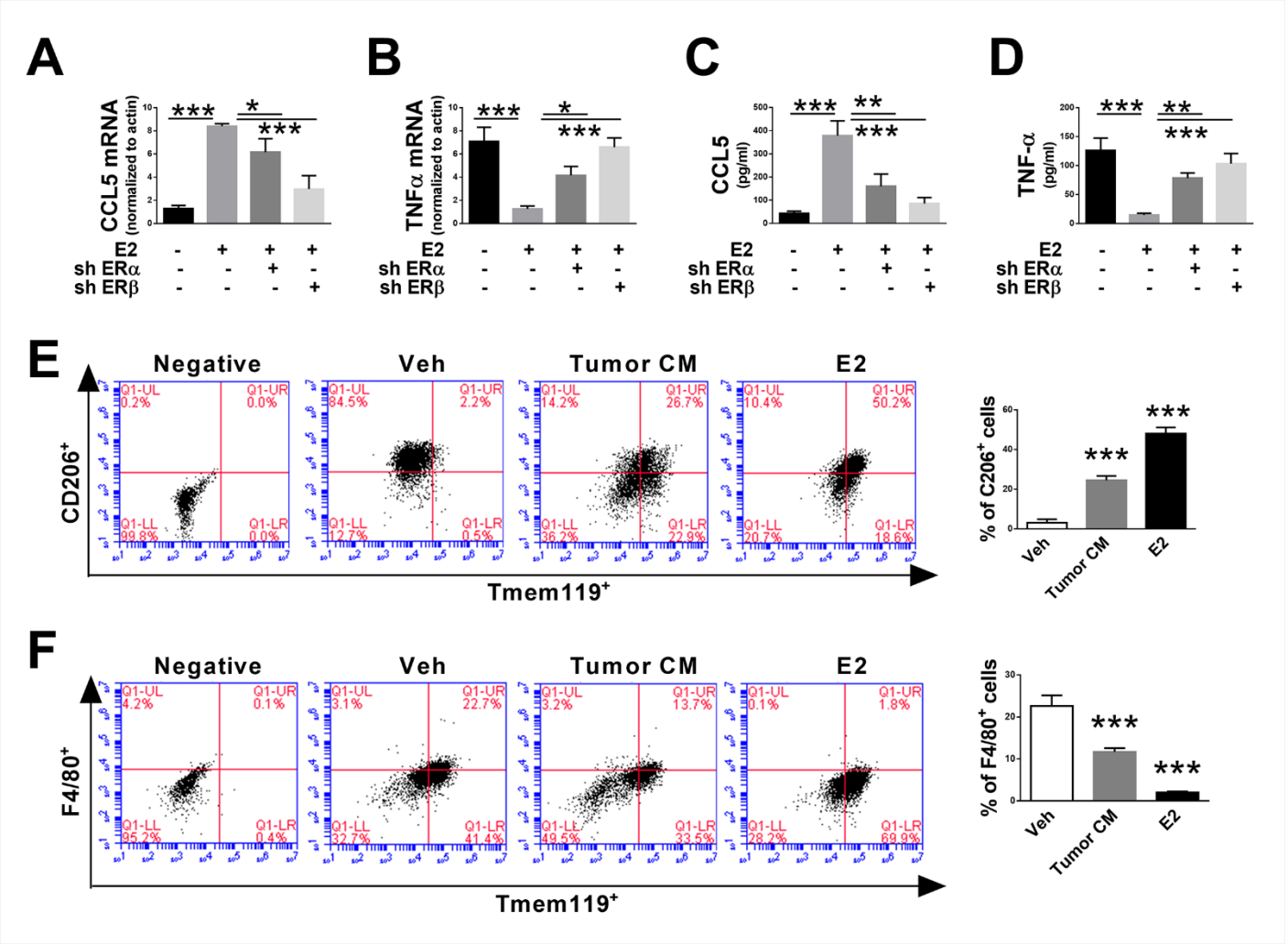
A. Human microglia (HMC3) cells were incubated with the DMEM/F-12 no phenol red medium (PRF F12) which contained with 2% fetal bovine serum (FBS) or HMC3 cells were incubated with regular DMEM/F-12 which contained with 2% charcoal-stripped FBS (CS FBS) for 24 hours and then examined for cell viability. *t* test, **: $p < 0.01$; ***: $p < 0.001$. ($n = 3$). **B, C.** Effect of (B) M1 ($F4/80^+$) and (C) M2 ($CD206^+$) polarization on HMC3 microglia cells in the presence of phenol red free culture medium or charcoal-stripped fetal bovine serum treatment. The data are presented as the mean ± SD. One-way ANOVA: no significantly. ($n = 3$).



Supplementary Fig 4: Blocking estrogen receptors (ERs) on microglia suppressed E2-derived microglia condition medium (CM)-mediated stemness.

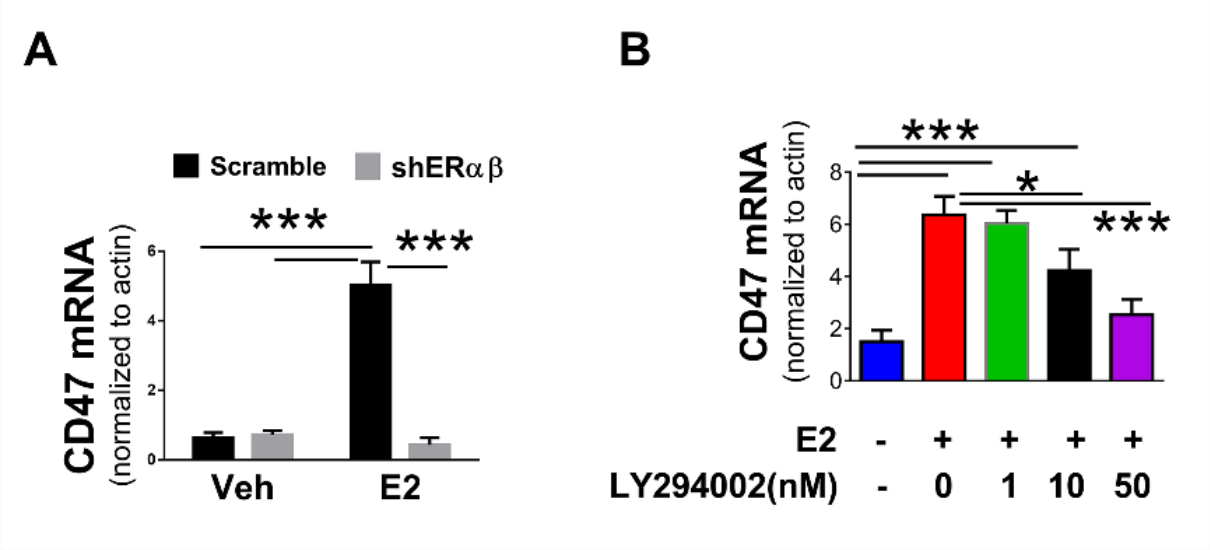
A, B. SKBrM (A) and 231BrM (B) cells were treated with or without E2 (1 nM) for 24 hours followed by measuring the stemness genes by qRT-PCR. **C, D** Effects of the CM derived from E2-treated microglia on cancer stem cell markers, $CD44^{+}$/ ESA^{+}, in SKBrM (C) and in 231BrM (D) were examined by FACS. **E, F.** 231BrM cells were incubated with the CM derived from E2-treated microglia which exposed with or without shERα/β treatment. 231BrM cells were incubated with CM for 24 hours and then examined for colony ability (E) and cell viability (F). Microglia CM: human microglia (HMC3) cells in the presence or absence of shERα/β were

treated with PBS or E2 (1 nM) for 24 hours, and they were washed with PBS and incubated in the fresh DMEM/F12 medium supplemented with 2% FBS for 24 hours. **G**. Effect of the CM derived from E2-treated microglia in the presence or absence of shERα/β pretreatment on cancer stem cell markers, $CD44^{+}$/ ESA^{+} were examined using flow cytometry. **H, I**. 231BrM cells were incubated with the CM derived from E2-treated microglia in the presence or absence of shERα/β treatment for 24 hours, and the expressions of stemness genes were examined by qRT-PCR. The data are presented as the mean ± SD. One-way ANOVA, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. ($n = 3$).



Supplementary Fig 5: Effect of estrogen on cytokines production and microglia polarization in microglia cells after shERα/β or tumor condition medium (CM) treatment.

A-D. Human microglial (HMC3) cells in the presence or absence of shERα/β were treated with E2 (1 nM) for 24 hours and cells were washed with PBS twice and incubated in the fresh DMEM/F12 medium supplemented with 2% FBS for additional 24 hours. Cell lysates (A and B) and CM (C and D) were examined for the amount of CCL5 and TNF-α by qPCR and ELISA (n = 3/group). **E, F**. Effect of M1 ($F4/80^+/Tmem119^+$) and M2 ($CD206^+/Tmem119^+$) polarization on human microglia (HMC3) cells in the presence of tumor-derived CM or E2 (1nM) treatment. The data are presented as the mean ± SD. One-way ANOVA, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. (n = 3).



Supplementary Fig 6: Blocking ERs on tumor cells down-regulated the expression of CD47. **A.** 231BrM cells in the presence or absence of shERα/β were treated with or without E2 for 24 hours, and the expression of CD47 was examined by qRT-PCR. **B.** 231BrM cells in the presence or absence of different concentration of PI3K inhibitor, LY294002, were co-treated with or without E2 for 24 hours, and the expression of CD47 was examined by qRT-PCR. The data are presented as the mean ± SD. One-way ANOVA, *: $p < 0.05$; ***: $p < 0.001$. ($n = 3$).